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(57) Abstract

A microcapsule comprising a payload entrapped in liposomes, the liposomes being encapsulated in a hydrocolloid matrix, wherein the matrix comprises an alginate salt and gelatin or wherein the payload comprises a nutritional or pharmacologically active component and the liposomes comprise at least one nutritional or pharmacologically active lipid. The microcapsules or a formulation thereof may be used in medicine and to deliver part or all of the dietary requirements of animals in closed system aquaculture.

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MICROCAPSULES

The present invention relates to a new type of microcapsule for use in the delivery of solutes.

The controlled delivery of substances from microscopic depots has been the object of a wide spectrum of industrial and biomedical research. A great variety of pharmacological dosage forms have been developed (reviewed by Rogers, 1982) to achieve prolonged or controlled action of incorporated or encapsulated drugs.

Liposomes have been heralded as an exciting solution to various drug delivery problems. They are lipid-water systems in which an aqueous volume is enclosed by a bilayer of amphipathic lipids. Lipid bilayers are themselves well structured two-dimensional solutions, consequently, dissolution of liposomes and loss of their payload occurs when liposomes are exposed to hydrodynamic shear, lipolytic enzymes and emulsifiers. Hydrophilic solutes may be entrapped within their enclosed volume, but their capacity to transport hydrophobic solutes is limited. Among the further defects of liposomes which have considerably limited their application as controlled-release vehicles are their instability, variable leakage rates and propensity toward fusion, aggregation and precipitation. Despite enormous research efforts over the

last 20 years, these limitations have serverly restricted the practical application of liposomes for controlled release.

Alternative, non-liposomal, microcapsules and microparticles (including "nano-particles" (Kreuter, 1983)) have been prepared from a variety of macromolecular colloids in which the active principles are dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached. Restrictions in the applications of microcapsules derive from toxicity of the monomer and/or polymer, contamination of the polymer by monomer, free-radical initiators and catalysts, extraction and/or denaturation of the payload by an organic phase, chemical modification of the payload during polymerisation, and variations in biodegradation.

It has long been the goal of aquaculturists to develop a micro-encapsulated diet. When aquatic animals are cultured in closed systems, there is a delicate balance between proper feeding and over-feeding. In the latter instance, excess food leads to bacterial fouling of the culture water, which in turn, leads to loss of the animals. Microencapsulation techniques permit diet delivery and control without the problem of fouling. Many types of materials are available for the preparation of microcapsules and microparticles.

The first use of microencapsulation techniques as an approach to producing artificial diets was as recent as 1974 when Jones et al. modified the original technique of Chang et al. (1966) for producing nylon-protein microcapsules. Nylon-protein microcapsules have been fed to a variety of organisms, including: larvae of the brine shimp, Artemia (Jones et al. 1974; Jones et al. 1975; Jones and Gabbott, 1976); the shrimps, Macrobrachium rosenbergii, Crangon crangon, Palaemon elegans and Palaemons merguiensis and the hermit crab, Pagurus bernhardus (Jones et al. 1975); the shrimp, Penaeus japonicus (Jones et al. 1979a, 1979b); larvae of the oyster, Crassostrea virginica (Chu et al. 1982); the oyster, Crassostrea gigas (Gabbott et al., 1976; Langdon, 1977) and juveniles of the blue mussel, Mytilus edulis (Gabbott et al., 1976).

These results clearly demonstrate that nylon-protein microcapsules may be utilised for dietary supplementation in aquacultural systems. However, there are major disadvantages with this type of microcapsule. Only macromolecular dietary components can be encapsulated and retained in the capsule due to the high permeability of the capsule wall (Jones and Gabbott, 1976; Jones et al. 1976), and, large amounts of lipid and hydrophobic components are lost from the aqueous diet phase to the

organic solvent during the encapsulation process (Jones and Gabbott, 1976; Jones et al. 1979a).

Langdon and Waldock (1981) used gelatin-ethyl cellulose microcapsules to encapsulate carbohydrate in their studies of bivalve nutrition. Although these capsules are well suited for specific applications, their utility is limited. Ethyl cellulose microcapsules can be used only for encapsulating carbohydrate and protein (aqueous nutrients) and can not be used to encapsulate lipid because the technique requires the use of organic solvents, which remove the hydrophobic components from the microcapsules.

Gelatins are commonly used as binders in aquatic animal feeds (New, 1976), either singly or in mixtures. The bound material is crushed into suitably sized pieces for use. The pieces break up quickly in aqueous environments and tend to foul the water in a matter of hours. The use of gelatins for preparing microencapsulated or microparticulate diets for aquacultural purposes, however, began as recently as 1981 when Langdon and Waldock first used gelatin-acatia to encapsulate neutral oils.

The utility of gelatin-acatia as a matrix for diet delivery is restricted because only non-aqueous nutrients (e.g. lipids) may be entrapped. The gel forms

semi-solid particles of a porous matrix which can not be used for the encapsulation of a total diet containing protein, carbohydrate and lipid because the aqueous elements are partially lost during preparation.

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Calcium alginate gels have been used widely in the chemical and food industries as thickeners and to control texture. Published studies (Levine and Sulkin, 1984a,b) have demonstrated the versatility of calcium alginate microcapsules for encapsulating dietary particles and/or oils. Microcapsules of calcium alginate may be enriched with specific polyunsaturated fatty acids. The entrapped fatty acids remain tightly associated with the capsules and do not desorb nor escape at an appreciable rate. The calcium alginate system can be used to encapsulate powder and lipid simultaneously provided the powder is emulsified in the lipid.

Levine and co-workers have used calcium alginate microcapsules to study the nutritional requirements of brachyuran crab larvae (Levine, 1983, Levine et al. 1983, Levine and Sulkin, 1984a,b). "Empty" microcapsules are not nutritious in and of themselves. Survival and development rates of crab larvae were improved when a diet of live rotifers, Brachionus plicatilis, was supplemented with calcium-alginate microcapsules containing dietary additions. When the rotifer diet was fed in combination with encapsulated unsaturated fatty acids, ingestion of microcapsules resulted in assimilation of single microencapsulated long chain polyunsaturated fatty acids into megalopa (postlarval stage) total lipid.

These experiments collectively demonstrate that encapsulation of specific dietary components in calcium alginate microcapsules results in assimilation by crab larvae. Due to their high permeability, however, these microcapsules can not be used to encapsulate water-soluble components in solution (e.g. vitamins, amino acids, enzymes etc.)

All of the above mentioned gelling-hydrocolloid techniques share a common disadvantage, namely limited ability to produce a gelatin microcapsule that will retain water-soluble nutrients. Furthermore, because each type of microcapsule, with the exception of the calcium alginate system developed by Levine and co-workers, is specialised for encapsulating a specific dietary component (e.g. lipid or carbohydrate), no single method can be used to produce a complete artificial diet.

Langdon and co-workers (Langdon, 1983; Langdon and Siegfried, 1984; Langdon and Bolton, 1984) and Chu et al. (1983) have developed "lipid-walled" capsules specifically for encapsulating water-soluble nutrients. These capsules are composed of neutral lipids and must be fed with other types of microcapsules since they alone can not be used to deliver a complete diet. Langdon and co-workers have also developed a "microgel particle" that is similar to the calcium alginate microcapsule described

by Levine (1983) except that they are produced by a solvent evaporation process rather than by spraying (Langdon and Levine, 1983; Langdon et al., 1985). These particles include a phospholipid mixture (soy lecithin) as a dietary supplement.

Wheatley et al. (1985) has described a drug delivery system wherein prolonged release of myoglobin is achieved using a liposomal formulation of myoglobin entrapped in a matrix of alginate cross-linked by calcium ions.

It has now been discovered that pharmacologically active agents or nutritional components can be delivered by encapsulation in liposomes comprising nutritionally or pharmacologically active lipids, the liposomes being protected by microencapsulation in a

hydrocolloid matrix and that certain hydrocolloid matrices containing alginate and gelatin offer particular advantages in ease of encapsulation and control in release of nutritional or other payloads from entrapped liposomes.

Accordingly the present invention provides a microcapsule comprising a payload entrapped in liposomes, the liposomes being encapsulated in a hydrocolloid matrix, wherein the matrix comprises an alginate salt and gelatin or wherein the payload comprises a nutritional or pharmacologically active component and the liposomes comprise at least one nutritional or pharmacologically active lipid.

Preferably the microcapsule comprises a payload, comprising a nutritional or pharmacologically active component, entrapped in liposomes comprising at least one nutritional or pharmacologically active lipid and the liposomes are encapsulated in a matrix comprising alginate and gelatin.

Hereafter the term "lipogel microcapsule" will be used to denote a microcapsule according to the invention. Those lipogel microcapsules wherein the matrix comprises an alginate salt and gelatin are designated Type I lipogel microcapsules and those wherein the payload is a nutritional or pharmacologically active component and the liposomes comprise at least one nutritional or pharmacologically active lipid are

designated Type II lipogel microcapsules. However it should be understood that the preferred lipogel microcapsules of the invention embody the features of both Type I and Type II lipogel microcapsules and thus that these terms are not mutually exclusive but involve considerable overlap.

As regards Type I lipogel microcapsules, the payload may be any hydrophilic or hydrophobic solute (excluding those which would solubilize the lipid bilayer) which can be entrapped in liposomes and the liposomes may be composed of one or a mixture of lipids selected to entrap the payload and provide desired release characteristics. Here it is the use of alginate/gelatin as matrix which is particularly important.

The composition of the entrapped liposomes may be controlled to achieve controlled release of the payload as a function of temperature, pH, or ion concentration. The liposome may be composed of any bilayer- or micelle-forming lipid. All lipids in these catagories may be suitable, regardless of their combined or individual permeability, since they may be combined to achieve the desired payload-release kinetics. Permeation of the payload through the wall of the microcapsule may be regulated by the ratio of alginate to gelatin, their absolute concentrations in the microcapsule, the extent of cross-linking of the microcapsular matrix, and the chemistry of cross-linking.

The composition of the liposomal components of Type I Lipogel Microcapsules may be varied to suit specific applications. Liposomes may be prepared from essentially any class of phospholipid; variability in phospholipid class derives from each of the hydrophobic and hydrophilic domains of these amphipathic molecules. Individual phospholipids may be chosen based upon their permeability and release characteristics. Permeability of individual liposome preparations is dependent upon: extent of unsaturation, presence or absence of sterols, the temperature of their gel-liquid-crystalline phase transition, the ionic- and pH-dependance of their lamellar-hexagonal transition, the temperature-dependence of non-bilayer configurations, and the extent of monomer conversion of polymerizable phospholipids (Hayward et al., 1985).

In addition to composition, the configuration of liposomal preparations may be varied to suit specific applications. A great variety of liposomal preparatory methods are available which differ in: ease of production, solvent and equipment requirements, and tolerance for different lipid classes. The liposomes produced by these different methodologies vary in: number of lamellae, aqueous volume enclosed per mole of phospholipid, resistance to shear, entrapment efficiency, diameter, and

resistance to fusion/aggregation/precipitation (reviewed by Gregoriadis, 1984). The choice of liposomal configuration will be dictated largely by the intended application and the required profile of payload release.

Suitably the matrix comprises an alginate salt and gelatin in a ratio of from 99:1 to 1:99 by weight, preferably from 10:1 to 1:1 and most preferably about 3:1 by weight. The ratio of alginate to gelatin may be modified to modulate the permeability barrier posed by the matrix. Various soluble and insoluble salts of alginate may be employed; preferred soluble salts include sodium alginate and preferred insoluble salts include calcium alginate.

Without wishing to be bound by any theory it is believed that the gelatin content of the microcapsules may serve to modify the permeability of the capsular wall. In addition, the simultaneous presence of gelatin and

alginate permits the formation of alginate-alginate, alginate-protein and protein-protein cross-links through the use of chemical cross-linkers such as formaldehyde.

In one application of Type I lipogel microcapsules, the payload is a pharmacologically active substance, such as a drug. Type I lipogel microcapsules may be designed to deliver the drug to a particular locus of action by exploitation of specific administrative routes, such as oral, topical, opthalmic, rectal, vaginal, subcutaneous and other parenteral routes. Among many other uses Type I lipogel microcapsules may also be used to deliver flavour or aroma substances and in the adhesives industry.

Encapsulation of liposomes within a matrix of alginate/gelatin yields a carrier-vehicle which is fully biodegradable, non-toxic and capable of transporting both hydrophilic and hydrophobic solutes. The temperature-dependent release of flavours and aromas can be achieved by selection of a phospholipid, or other glyceride or fatty acid, which exhibits a temperature-dependent change in permeability and/or configuration. Similar systems may be designed in which the stimulus for a change in permeability is an alteration in pH, ionic strength or light intensity.

In the adhesives industries, an example of the use of Type I lipogel microcapsules is the inclusion of initiators or catalysts within the entrapped volume of liposomes, which are themselves entrapped within

the matrices of alginate/gelatin particles, which are in turn dispersed in a polymerisable solution or adhesive. Physical destruction of the microcapsule, as achieved by cavitation or similar shock, releases the initiator which in turn converts monomer to polymer or initiates the curing of adhesives.

Turning to Type II lipogel microcapsules, the payload may be any pharmacologically active or nutritional component or a mixture of such components whilst the liposomes are formed of at least one nutritional or pharmacologically active lipid. The term "nutritional component" encompasses all materials which are nutritionally valuable as a constituent part of an organism's diet, whether as an essential component of that diet or otherwise. This includes components of specialised diets which may be used in treating particular disease or deficiency states. Thus examples of nutritional components are proteins, minerals, carbohydrates, vitamins and amino acids. The liposomes are formed from at least one lipid which is either a nutritional component or which has a pharmacological activity in its own right. Examples of the former are the unsaturated fatty acids required as part of the diet fed to aquatic organisms in aquaculture. Because of the unique encapsulation system of a matrix encapsulating liposomes entrapping nutritional components it is, for the

first time, possible to produce a complete diet for an organism in a single formulation. This may take the form, for instance, of a formulation for intravenous feeding of a human patient while the patient is prevented by medical or other circumstances from taking food and drink by mouth or otherwise through the alimentary canal. Alternatively it may take the form of a complete diet for use in aquaculture. Preferably the payload and liposome of a Type II lipogel microcapsule constitute a complete diet for an organism such as a mollusc, crustacean, fish or mammal especially man.

Polyunsaturated fatty acids and sterols must be supplied in aquacultural diets because of the inability of larvae to synthesise these compounds de novo. Normally, these exogenous lipids are supplied in multicomponent diets. Provision of the appropriate unsaturated lipids in a single-capsule diet may be attained using a phospholipid fraction of fish oil such as dogfish oil or menhaden oil, obtained from commercial fish processing plants and isolated by column chromatography (Bartlett, 1959), that is enriched in polyunsaturated fatty acid. Permeability of liposomes derived from polyunsaturated menhaden phospholipids may be regulated by the addition of increasing mole fractions of cholesterol, itself an obligatory component of the aquacultural diet. Oxidation of the unsaturated lipids may be inhibited by the inclusion of commercially employed antioxidants such

as alpha-tacopherol (vitamin E), butylated hydroxy toluene (BHT), ascorbic acid (vitamin C), nordihydroguaiaretic acid and others. The specified antioxidant may be chosen to enhance the nutritional value of the diet.

Suitably Type II microcapsules have a hydrocolloid matrix or wall that is digestible, that retains water-soluble nutrients, and that permits the delivery of a complete diet. Types of colloids employed for generation of microcapsules include the gelling hydrocolloids such as agar, algin, carrageenan, carboxymethyl cellulose, furcellaran, gelatin, pectin and xanthan, suitable polymerised organics, and the products formed by chemical cross-linking of natural materials such as human and bovine albumin, casein and gelatin. Combinations of different colloidal types, such as the acrylic dextrans, yield carriers which exhibit characteristics of each of the components.

In both Type I and Type II lipogel microcapsules the liposomes may be uni- or multilamellar, and they may be of any suitable size appropriate to the intended use of the lipogel microcapsules and the materials used to form the liposomes. Suitable lipid compositions for the liposomes used in Type I and Type II lipogel microcapsules include mixtures of lecithin (e.g. soy lecithin) or

dipalmitoylphosphatidyl choline (DPPC) and cholesterol in a molar ratio of from 200:1 to 1:2, for instance 2:1 to 1:2, preferably about 1:1.

Lipogel microcapsules may be formed in any size appropriate to their intended use, though typically they will be from 0.1 nm to 5 nm or more in mean diameter (it is usual for there to be a spread of diameters centering on the desired mean value). For intravenous use as a pharmacological or nutritional agent the lipogel microcapsules would typically have a microscopic particle size, cosmetics formulations could have particle sizes perhaps up to 1 mm whereas subcutaneous implants, suppositories etc. may be up to a few centimetres in size as would aquaculture particles. A large number of liposomes will be encapsulated by the hydrocolloid matrix of each lipogel microcapsule.

The present invention also provides a process for producing lipogel microcapsules which process comprises

a) entrapping the payload in liposomes

and b) encapsulating the liposomes in a hydrocolloid matrix.

In step a) the liposomes may be formed by conventional processes such as those described by Gregoriadis (1984).

Step b) is accomplished by admixing the liposomes with an aqueous solution of the hydrocolloid matrix material. Curing or polymerization of the microcapsular matrix may be accomplished by chemical means (as in the calcium ion mediated precipitation of sodium alginate) or by physical means (as in the thermal gelling of the carrageenans). The desired particle size may be achieved by curing an atomised dispersion of the monomeric hydrocolloid, by extrusion methods, or by curing in bulk followed by crushing.

In a preferred embodiment of this process the payload is entrapped in the liposomes by reverse phase evaporation and the liposomes are then separated from unentrapped material and washed. The liposomes are then admixed with the hydrocolloid matrix material in aqueous solution and sprayed into a curing bath. Conveniently liposomes comprising a phospholipid are aerosolised or nebulised using conventional nebulising or aerosol-forming equipment and a suitable carrier gas such as compressed air. In this way droplets of matrix solution each containing a number of liposomes are formed and the matrix is then cured in the curing bath. When forming Type I lipogel microcapsules the curing bath preferably contains calcium chloride solution, for instance at a concentration in the range of from about 1% to about 20% w/v and the

lipogel microcapsules reside in the curing bath for about 5 minutes in order to harden the alginate/gelatin matrix. The viscosity and texture of the lipogel microcapsules is dependent upon the extent (e.g., duration) of curing the concentration and type of curing agent, the concentration of alginate, and the ratio of gelatin to alginate. After formation and curing, the lipogel microcapsules are separated from the curing bath and washed prior to storage or use. Suitably storage is as a suspension in distilled water with added chloramphenicol or other antimicrobial agent. Preferably the lipogel microcapsules are stored under nitrogen at about 10°C. Alternatively the lipogel microcapsules may be made up into the desired end product, for instance a pharmaceutical formulation or aquacultural diet by admixture with an appropriate carrier and optional accessory ingredients. As a further alternative, in some preparations, the lipogel microcapsules may be stored as a freeze-dried powder wherein the structural integrity of the microcapsule is maintained by the presence of high levels of carbohydrate.

The present invention therefore also provides a formulation comprising lipogel microcapsules and a carrier or diluent therefor. In a particular embodiment the invention provides a formulation comprising lipogel microcapsules suspended in a continuous aqueous or oily

phase containing at least one emulsifier or surfactant. Examples of such formulations include suspensions of lipogel microcapsules in oils and in creams, lotions, gels and emulsions for instance for topical cosmetic or pharmacological treatment. Suitably lipogel microcapsules will comprise from about 0.001% by weight of such formulations, preferably 1-10% by weight.

The invention also provides a process for producing formulations described above comprising bringing into association lipogel microcapsules and a carrier or diluent therefor.

The invention further provides lipogel microcapsules or formulations thereof for use in the treatment of the human or animal body and a method for treating the human or animal body comprising administration of a sufficient amount of lipogel microcapsules or a formulation thereof.

The invention also provides a foodstuff comprising Type II lipogel microcapsules as hereinbefore defined and a method for feeding livestock wherein the livestock are provided with Type II lipogel microcapsules or a formulation thereof in partial or complete fulfilment of the dietary requirement of the livestock.

The invention will now be illustrated by the following Examples which are not intended to limit the invention in any way.

EXAMPLE 1

(a) Production of Liposomes

Liposomes were constructed using cholesterol and (a) soy lecithin or (b) dipalmitoylphosphatidyl-choline (DPPC)(1:1, mole:mole). The lipid was initially dissolved in chloroform (5 ml) in a 50 ml conical flask and dried to a thin film by evaporation under nitrogen. A solution of 6-carboxyfluorescein (6-CF; Eastman 0.25 M) was prepared in distilled water and the pH was adjusted to 7.4 using sodium hydroxide (Senior and Gregoriadis, 1984). This dye is self-quenching at this concentration and is used as an aqueous marker for liposome leakage. Additionally, 6-CF may be considered as an analogue for biomedical or industrial payloads. 6-CF solution (25 ml) was added to the lipid and the conical flask was heated to 40°C. The 6-CF-lipid mixture was flushed with nitrogen for 3 minutes, 3 glass beads were added, the flask was stoppered, and mixed with a vortex mixer for 3 minutes to form multilamellar vesicles (MLV's). The suspension of MLV's was sonicated using a bath sonicator (Laboratory Supplies, N.Y.) for 15 minutes. The liposomes were separated from unentrapped material by passing the suspension through a Sepharose CL-6B (Pharmacia) column (1 cm x 20 cm) (Senior and Gregoriadis, 1984).

b) Production of Type 1 lipogel microcapsules

Liposomes prepared as described above were entrapped in calcium alginate-gelatin as follows. The pH of distilled water was adjusted to 12.00 by the dropwise addition of sodium hydroxide (10 M). Sodium alginate (1.6% w/v) and gelatin (0.5% w/v) were added and the mixture stirred on a combination hot and stir plate at 40°C until completely dissolved. Following the readjustment of pH to 7.4, the liposomes were stirred into the alginate-gelatin mixture using a stir bar (The temperature of the combined dispersions may be adjusted to account for the thermal tolerance of the liposomes). After the liposomes were added to the alginate-gelatin polymer, the mixture was poured into a Pyrex thin-layer chromatography atomizer (e.g., SMI spray atomizer), and sprayed into a curing bath of calcium chloride (150 ml, 20% w/v) in a 1 liter beaker. Compressed air (at approximately 276 kPa to 414 kPa, 40-60 psi) may be used when encapsulating materials not sensitive to oxidation but when encapsulating polyunsaturated fatty acids or other substances prone to oxidation, compressed nitrogen or argon should be used. The microcapsules were hardened in the calcium chloride for 5 min and collected on a 44 um mesh sieve. The microcapsules were rinsed on

the sieve with distilled water and stored in a chloramphenicol solution in distilled water (5 mg/l), under nitrogen at 10°C. The addition of chloramphenicol is necessary to prevent bacterial growth. This step may be omitted if the microcapsules are employed immediately or if an alternative method of sterilisation (e.g., irradiation) is available. The liposomes were encapsulated at up to 20% by weight of the total material. The latency (% of total aqueous solute contained within the liposome) of liposomes was not affected by aerosolisation. Entrapment of the dye was immediately evident in the colour of the Lipogel Microcapsules.

c) Production of Type II Lipogel Microcapsules

Liposomes produced according to part (a) above but containing aqueous dietary components such as vitamins are encapsulated in alginate gelatin according to the method of part (b) above.

CLAIMS

1. A microcapsule comprising a payload entrapped in liposomes, the liposome being encapsulated in a hydrocolloid matrix, wherein the matrix comprises an alginate salt and gelatin or wherein the payload comprises a nutritional or pharmacologically active component and the liposomes comprise at least one nutritional or pharmacologically active lipid.

2. A microcapsule according to claim 1 wherein the matrix comprises an alginate salt and gelatin.

3. A microcapsule according to claim 2 wherein the matrix comprises an alginate salt and gelatin in a weight ratio of 99:1 to 1:99.

4. A microcapsule according to claim 3 wherein the matrix comprises an alginate salt and gelatin in a weight ratio of 10:1 to 1:1.

5. A microcapsule according to claim 3 wherein the matrix comprises an alginate salt and gelatin in a weight ratio of about 3:1.

6. A microcapsule according to any one of claims 2 to 5 wherein the alginate salt is sodium or calcium alginate.

7. A microcapsule according to any one of claims 2 to 6 wherein the matrix is cross-linked.

8. A microcapsule according to claim 7 wherein the matrix is cross-linked by treatment with formaldehyde.

9. A microcapsule according to any preceding claim wherein the payload comprises a compatible hydrophilic or hydrophobic solute.

10. A microcapsule according to claim 9 wherein the payload is a pharmacologically active substance, a flavour or aroma substance or an adhesive component.

11. A microcapsule according to any one of claims 1 to 9 wherein the payload comprises a nutritional or pharmacologically active component and the liposomes comprise at least one nutritional or pharmacologically active lipid.

12. A microcapsule according to claim 11 wherein the payload is a protein, mineral, carbohydrate, vitamin or amino acid or a mixture of at least two thereof.

13. A microcapsule according to claim 11 or claim 12 wherein the matrix is a gelling hydrocolloid selected from agar, algin, carrageenan, carboxymethyl cellulose, furcellaran, gelatin, pectin, xanthan, dextran, organic polymers and chemically cross-linked albumin, casein and gelatin and mixtures of at least two thereof.

14. A microcapsule according to any proceding claim wherein the liposomes comprise at least one unsaturated fatty acid, polyunsaturated fatty acid or sterol.

15. A microcapsule according to claim 14 wherein the liposomes comprise at least one polyunsaturated menhaden phospholipid.

16. A microcapsule according to claim 14 or claim 15 wherein the liposomes comprise cholesterol.

17. A microcapsule according to any one of claims 14 to 16 wherein the liposomes comprise (a) lecithin or dipalmitoyl phosphatidyl choline and (b) cholesterol in a molar ratio of 200:1 to 1:2.

18. A microcapsule according to claim 17 wherein the liposomes comprise (a) lecithin or dipalmitoyl phosphatidyl choline and (b) cholesterol in a molar ratio of 2:1 to 1:2.

19. A microcapsule according to claim 18 wherein the liposomes comprise (a) lecithin or dipalmitoyl phosphatidyl choline and (b) cholesterol in a molar ratio of about 1:1.

20. A microcapsule according to any one of claims 17 to 19 wherein the liposomes comprise soy lecithin.

21. A process for producing a microcapsule according to any preceding claim comprising (a) entrapping the payload in liposomes and (b) encapsulating the liposomes in a hydrocolloid matrix.

22. A process according to claim 21 comprising

the further step of curing the microcapsules.

23. A process according to claim 21 or claim 22 wherein liposomes are admixed with an aqueous solution of the hyrocolloid matrix material and the suspension is nebulised or aerosolised using a suitable carrier gas and sprayed into a curing bath.

24. A formulation comprising microcapsules according to any one of claims 1 to 20 and a carrier or diluent therefor.

25. A formulation according to claim 24 wherein the carrier is an aqueous or oily phase containing at least one emulsifier or surfactant.

26. A microcapsule according to any one of claims 1 to 20 or a formulation according to claim 24 or claim 25 for use in the treatment of the human or animal body.

27. A method for feeding livestock comprising providing the livestock with microcapsules according to any one of claims 11 to 20 or a formulation thereof in partial or complete fulfilment of the dietary requirement of the livestock.

28. A method according to claim 27 wherein the livestock are aquatic animals cultured in a closed system.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00550

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

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II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0140085 (FUJISAWA PHARM. CO.) 8 May 1985 see page 4, lines 19-26; 23-25; claims	1,9-13,21, 23-28
X	DE, A, 2640707 (DR. THEUREN) 16 March 1976 see the whole document	1-6,9-28
X,Y	WO, A, 85/03640 (THE LIPOSOME CO.) 29 August 1985 see page 8, line 19 - page 9, line 18; page 13, lines 20-34; page 14, lines 22-33; page 20, lines 5-15; claims	1-28
Y	EP, A, 0133391 (GUERBET S.A.) 20 February 1985 see page 2, line 22 - page 3, line 32 page 5, lines 4-14; claims	1-6,9-28
P,X	EP, A, 0160266 (TERUMO CORP.) 6 November 1985	./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th December 1986	22 JAN 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where apropriate, of the relevant passages	Relevant to Claim No
	see page 3, line 36 - page 4, line 24; page 6, lines 19-30; page 9, lines 28-35; claims	1-28
	--	
P,X	EP, A, 0162724 (VESTAR RESEARCH) 27 November 1985 see claims	1-6,9-28
	--	
P,A	WO, A, 86/01404 (A. MIXSON) 13 March 1986 see claims	1,11-12,14, 26-28

FURTHER INFORMATION CONTINUED FROM THE SEC ND SHEET

PARTIALLY

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers §§..... because they relate to subject matter not required to be searched by this Authority, namely:

§§ Claims 27, 28
See Rule 39.1(iv):
Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 86/00550 (SA 14527)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/01/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0140085	08/05/85	JP-A-	60058915	05/04/85
DE-A- 2640707	16/03/78	None		
WO-A- 8503640	29/08/85	EP-A-	0172907	05/03/86
		JP-T-	61501686	14/08/86
EP-A- 0133391	20/02/85	FR-A,B	2548902	18/01/85
		JP-A-	60075436	27/04/85
EP-A- 0160266	06/11/85	JP-A-	60231609	18/11/85
EP-A- 0162724	27/11/85	JP-A-	60258109	20/12/85
		AU-A-	4285685	12/12/85
WO-A- 8601404	13/03/86	AU-A-	4779285	24/03/86
		EP-A-	0190343	13/08/86

For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82